

CLAIMS

[00175] We claim

[00176] 1. A microfluidic reaction device comprising:

[00177] (a) a plurality of chambers having a first conduit and a second conduit;

[00178] (b) a first transport channel having a first end, said first transport channel having a bypass channel at said first end, said first transport channel being in flow communication with at least one said chamber through connection with said first conduit;

[00179] (c) a second transport channel having a first end, said second transport channel having a bypass channel at said first end, said second transport channel being in flow communication with at least one said chamber through connection with said second.

[00180] 2. The microfluidic reaction device in claim 1, wherein interior surfaces of said first transport channel comprise a hydrophobic film.

[00181] 3. The microfluidic reaction device in claim 1 further comprises one or more distribution channels.

[00182] 4. The microfluidic reaction device in claim 1 further comprises oil in said first and second transportation channels and aqueous solution in said chambers.

[00183] 5. The microfluidic reaction device in claim 1 further comprises gas in said first and second transportation channels and aqueous solution in said chambers.

[00184] 6. The microfluidic reaction device in claim 1 further comprises beads in said chambers.

[00185] 7. A method for amplifying target nucleic acid comprising:

[00186] (a) attaching an oligonucleotide to a solid support within a chamber, the oligonucleotide comprising a first primer, a second primer and a binding probe sequence wherein the first primer, second primer and binding probe sequences are separated from one another and the solid support by a cleavable linker;

[00187] (b) incubating a target nucleic acid with the oligonucleotide under conditions in which complementary target sequence and binding probe sequence hybridize to one another;

[00188] (c) washing the chamber;

[00189] (d) adding a solution comprising a cleavage substance, polymerase, dNTPs, and divalent cation to the chamber such that the first primer, second primer and binding probe sequence are released from one another and from the solid support so that the first primer, second primer, binding probe sequence, target nucleic acid, polymerase, dNTPs and divalent cation produce a reaction mixture within the chamber;

[00190] (e) subjecting the reaction mixture to two or more cycles of heating and cooling such that the target nucleic acid is amplified.

[00191]

[00192] 8. The method of claim 7 wherein the target nucleic acid is DNA

[00193] 9. The method of claim 8 wherein the first primer, second primer and the binding probe sequence are DNA

[00194] 10. The method of claim 7 wherein the cleavable linker is selected from the group consisting of uridine and reverse uridine.

[00195] 11. The method of claim 7 wherein the oligonucleotide is attached to the solid support by a linker.

[00196] 12. The method of claim 7 wherein the cleavage substance is RNase A.

[00197] 13. A method for amplifying a plurality of target nucleic acids on a microarray wherein the microarray is comprised of a plurality of separate chambers comprising:

[00198] (a) attaching a first oligonucleotide to a solid support within a first chamber, the oligonucleotide comprising a first primer, a second primer and a first binding probe sequence wherein the first primer, second primer and binding probe sequence are separated from one another and the solid support by a cleavable linker;

[00199] (b) attaching a second oligonucleotide to a solid support within a second chamber, the second oligonucleotide comprising a third primer, a fourth primer and a second binding probe sequence wherein the third primer, fourth primer and second binding probe sequence are separated from one another and the solid support by a cleavable linker;

[00200] (c) incubating a target nucleic acid comprising two or more nucleic acid sequences with the first and second oligonucleotide under conditions in which complementary target nucleic acid sequences and binding probe sequences hybridize to one another;

[00201] (d) washing the chamber;

[00202] (e) adding a solution comprising a cleavage substance, polymerase, dNTPs, and divalent cation to the first and second chamber such that the first primer, second primer, third primer, fourth primer, first binding probe sequence and second binding probe sequence are released from one another and from the solid support so that the first primer, second primer, first binding probe sequence, target nucleic acid, polymerase, dNTPs and divalent cation produce a first reaction mixture within the first chamber and the third primer, fourth primer, second binding probe sequence, target nucleic acid, polymerase, dNTPs and divalent cation produce a second reaction mixture within the second chamber;

[00203] (f) subjecting the first and second reaction mixture to two or more cycles of heating and cooling such that a plurality of target nucleic acids are amplified.

[00204] 14. The method of claim 13 wherein the plurality of target nucleic acids are DNA.

[00205] 15. The method of claim 13 wherein the first second primer, third and fourth primer and the first and second binding probe sequences are DNA.

[00206] 16. The method of claim 13 wherein the cleavable linker is selected from the group consisting of uridine and reverse uridine.

[00207] 17. The method of claim 13 wherein the first and second oligonucleotides are attached to the solid support by a linker.

[00208] 18. The method of claim 13 wherein the cleavage substance is RNase A.

[00209] 19. The method of claim 13 wherein the first and second oligonucleotides are between 60 to 100 nucleotides long.

[00210] 20. The method of claim 13 wherein the polymerase is a thermostable DNA polymerase.

[00211] 21. A method for amplifying target nucleic acid comprising:

[00212] (a) synthesizing an oligonucleotide to a solid support within a chamber, the oligonucleotide comprising a first primer, a second primer and a binding probe sequence wherein the first primer, second primer and binding probe sequences are separated from one another and the solid support by a cleavable linker;

[00213] (b) incubating a target nucleic acid with the oligonucleotide under conditions in which complementary target sequence and binding probe sequence hybridize to one another;

[00214] (c) washing the chamber;

[00215] (d) adding a solution comprising a cleavage substance, polymerase, dNTPs, and divalent cation to the first and second chamber such that the first primer, second primer, and the binding probe sequence are released from one another and from the solid support so that the first primer, second primer, the binding probe sequence, target nucleic acid, polymerase, dNTPs and divalent cation produce a reaction mixture within the chamber;

[00216] (e) subjecting the reaction mixture to two or more cycles of heating and cooling such that the target nucleic acid is amplified.

[00217] 22. A method for amplifying a plurality of target nucleic acids on a microarray wherein the microarray is comprised of a plurality of separate chambers comprising:

[00218] (a) synthesizing a first oligonucleotide to a solid support within a first chamber, the oligonucleotide comprising a first primer, a second primer and a first binding probe sequence wherein the first primer, second primer and first binding probe sequence are separated from one another and the solid support by a cleavable linker;

[00219] (b) attaching a second oligonucleotide to a solid support within a second chamber, the second oligonucleotide comprising a third primer, a fourth primer and a second binding probe sequence wherein the third primer, fourth primer and second binding probe sequence are separated from one another and the solid support by a cleavable linker;

[00220] (c) incubating a target nucleic acid comprising two or more nucleic acid sequences with the first and second oligonucleotide under conditions in which complementary target nucleic acid sequences and binding probe sequences hybridize to one another;

[00221] (d) washing the chamber;

[00222] (e) adding a solution comprising a cleavage substance, polymerase, dNTPs, and divalent cation to the first and second chamber such that the first primer, second primer, third primer, fourth primer, first binding probe sequence and second binding probe sequence are released from one another and from the solid support so that the first primer, second primer, first binding probe sequence, target nucleic acid, polymerase, dNTPs and divalent cation produce a first reaction mixture within the first chamber and the third primer, fourth primer, second binding probe sequence, target nucleic acid, polymerase, dNTPs and divalent cation produce a second reaction mixture within the second chamber;

[00223] (f) subjecting the first and second reaction mixture to two or more cycles of heating and cooling such that a plurality of target nucleic acids are amplified.

[00224] 23. A method for amplifying target nucleic acid comprising:

[00225] (a) attaching a first primer, a second primer and a binding probe sequence to a solid support such that the first primer, second primer and binding probe sequence is attached to the solid support within a chamber such that when treated with a cleavage substance the first primer, second primer and binding probe sequence are released from the solid support;

[00226] (b) incubating a target nucleic acid with the oligonucleotide under conditions in which complementary target sequence and binding probe sequence hybridize to one another;

[00227] (c) washing the chamber;

[00228] (d) adding a solution comprising a cleavage substance, polymerase, dNTPs, and divalent cation to the chamber such that the first primer, second primer and binding probe sequence are released from the solid support so that the first primer, second primer, binding probe sequence, target nucleic acid, polymerase, dNTPs and divalent cation produce a reaction mixture within the chamber;

[00229] (e) subjecting the reaction mixture to two or more cycles of heating and cooling such that the target nucleic acid is amplified.

[00230] 24. A method for amplifying target nucleic acid comprising:

[00231] (a) synthesizing a first primer, a second primer and a binding probe sequence to a solid support such that the first primer, second primer and binding probe sequence are attached to

the solid support within a chamber such that when treated with a cleavage substance the first primer, second primer are released from the solid support;

[00232] (b) incubating a target nucleic acid with the oligonucleotide under conditions in which complementary target sequence and binding probe sequence hybridize to one another;

[00233] (c) washing the chamber;

[00234] (d) adding a solution comprising a cleavage substance, polymerase, dNTPs, and divalent cation to the chamber such that the first primer and second primer are released from the solid support so that the first primer, second primer, binding probe sequence, target nucleic acid, polymerase, dNTPs and divalent cation produce a reaction mixture within the chamber;

[00235] (e) subjecting the reaction mixture to two or more cycles of heating and cooling such that the target nucleic acid is amplified.

[00236] 25. A method for amplifying a plurality of target nucleic acids on a microarray wherein the microarray is comprised of a plurality of separate chambers comprising:

[00237] (a) attaching a first primer, a second primer and a first binding probe sequence are attached to the solid support within a first chamber such that when treated with a cleavable substance the first primer and second primer are released from the solid support;

[00238] (b) attaching a third primer, a fourth primer and a second binding probe sequence are attached to the solid support within a second chamber such that when treated with a cleavage substance the third primer and fourth primer are released from the solid support;

[00239] (c) incubating a target nucleic acid comprising two or more nucleic acid sequences with the first and second binding probe sequences under conditions in which complementary target nucleic acid sequences and binding probe sequences hybridize to one another;

[00240] (d) washing the chamber;

[00241] (e) adding a solution comprising a cleavage substance, polymerase, dNTPs, and divalent cation to the first and second chamber such that the first primer, second primer, third primer and fourth primer are released from the solid support so that the first primer, second primer, target nucleic acid, polymerase, dNTPs and divalent cation produce a first reaction mixture within the first chamber and the third primer, fourth primer, target nucleic acid,

polymerase, dNTPs and divalent cation produce a second reaction mixture within the second chamber;

[00242] (f) subjecting the first and second reaction mixture to two or more cycles of heating and cooling such that a plurality of target nucleic acids are amplified.

[00243] 26. A method for amplifying a plurality of target nucleic acids on a microarray wherein the microarray is comprised of a plurality of separate chambers comprising:

[00244] (a) synthesizing a first primer, a second primer and a first binding probe sequence is attached to the solid support within a first chamber such that when treated with a cleavage substance the first primer and second primer are released from the solid support;

[00245] (b) attaching a third primer, a fourth primer and a second binding probe sequence is attached to the solid support within a second chamber such that when treated with a cleavage substance the third primer and fourth primer are released from the solid support;

[00246] (c) incubating a target nucleic acid comprising two or more nucleic acid sequences with the first and second binding probe sequences under conditions in which complementary target nucleic acid sequences and binding probe sequences hybridize to one another;

[00247] (d) washing the chamber;

[00248] (e) adding a solution comprising a cleavage substance, polymerase, dNTPs, and divalent cation to the first and second chamber such that the first primer, second primer, third primer, fourth primer and first binding probe sequence are released from the solid support so that the first primer, second primer, target nucleic acid, polymerase, dNTPs and divalent cation produce a first reaction mixture within the first chamber and the third primer, fourth primer, target nucleic acid, polymerase, dNTPs and divalent cation produce a second reaction mixture within the second chamber;

[00249] (f) subjecting the first and second reaction mixture to two or more cycles of heating and cooling such that a plurality of target nucleic acids are amplified.